

the help of the bound nucleotide. Based on these simulations, we propose that the ATP and DNA binding enhance the binding of each other by an allosteric mechanism mediated by a series of residues between the ATP and DNA binding sites.

### 1315-Pos Board B225

#### **Distinct Conformational Changes of MutS during DNA Mismatch Repair** **Jong-Bong Lee, Cherlhyun Jeong, Won-Ki Cho, Changill Ban, Richard Fishel.**

It has been studied that DNA repair proteins search a target via a 1-dimensional diffusion along naked DNA. Due to the absence of the target on DNA this single-molecule tracking approach lacked of understanding the catalytic function of the repair proteins and moreover the mechanism of the downstream transactions for the repair of an error on the DNA. We present the catalytic processes of MutS, mismatch repair initiation protein, on a mismatched DNA that bears an unpaired nucleotide. Our single-molecule analysis reveals that MutS undergoes two distinct conformational changes during DNA mismatch repair (MMR). MutS forms a transient clamp that scans duplex DNA for the unpaired nucleotide and a different sliding clamp that is induced by ATP binding to the mismatch-bound MutS with unusual stability on DNA. These observations suggest a mechanism of how MMR machinery can be recruited for the strand incision, which is highly controversial.

### 1316-Pos Board B226

#### **Reconstitution of Bacterial Plasmid Segregation and its Dynamic Pattern Formation**

**Ling Chin Hwang, Yong-Woon Han, Anthony Vecchiarelli, Barbara Funnell, Kiyoshi Mizuuchi.**

The segregation of genetic material prior to cell division is an essential process for all organisms. In order to maintain faithful inheritance of genetic information, chromosome copies must be partitioned to the daughter cells before cell division. The bacterial P1 plasmid in *Escherichia coli* is a simple yet useful model system to study the mechanism of chromosome segregation in prokaryotes as they encode only three components that are necessary for plasmid partitioning: a *parS* DNA sequence on the plasmid acting as the partition site and two partition proteins, ParA and ParB. *In vivo* studies have shown that the dynamics of ParA, an ATPase, is involved in the positioning of the plasmid copies with ParB bound at *parS* sites prior to cell division, but how these three components work together to drive plasmid segregation remains unanswered [1]. To better understand the mechanism of P1 plasmid partitioning, we have previously characterized ATP-dependent DNA binding process of ParA [2]. In this work, we reconstituted the three-component partition system in a cell-free reaction. We present the spatio-temporal dynamics of the ParA/B/S partition system observed real-time using TIRF microscopy.

[1] Gerdes K, Howard M, Szardenings F, Cell. 2010; 141(6): 927-42.

[2] Vecchiarelli AG, Han YW, Tan X, Mizuuchi M, Ghirlando R, Biertümpfel C, Funnell BE, Mizuuchi K., Mol. Microbiol. 2010; 78(1): 78-91.

### 1317-Pos Board B227

#### **Annealing Helicase HARP: A Single Molecule Study**

**Iwijn De Vlaminck, Timur Yusufzai, Marijn T.J. van Loenhout, Iztok Vidic, Roland Kanaar, Joyce H.G. Lebbink, James T. Kadonaga, Cees Dekker.**

HepA related protein (HARP) is an annealing helicase capable of rewinding complementary ssDNA stabilized by the single stranded (ss) DNA-binding protein Replication Protein A (RPA) [1]. Mutations in HARP ATPase are responsible for Schimke immuno-osseous dysplasia (SIOD). Recent reports show that HARP is recruited to sites of DNA damage via a binding interaction with RPA and, put HARP functionally at the replication fork under circumstances of replicative stress [2].

Here, we use a magnetic-tweezers single-molecule assay to study mechanistic aspects of HARP annealing-helicase activity. In a first step of the single-molecule assay, hRPA-stabilized bubbles of ssDNA are formed in a dsDNA molecule via hRPA-induced unwinding of dsDNA. hRPA locally unwinds double stranded DNA by binding and stabilizing bubbles of ssDNA, a process that relies on torsional energy present in the DNA [3]. Subsequently, we use the hRPA-stabilized bubbles of ssDNA as a starting substrate for studying the annealing helicase activity of HARP. We find that HARP is capable of complete rewinding of hRPA-bound bubbles of ssDNA. We confirm that the annealing helicase activity is ATP-dependent. The recorded reaction rates are proportional to [HARP] and sensitive to [KCL] with slower reactions occurring at higher [KCL]. Our results suggest that HARP, rather than actively displacing hRPA, functions as a ratchet that displaces hRPA by preventing rebinding of dissociated hRPA DNA-binding domains.

[1] T. Yusufzai et al. Science (2008).

[2] R. Driscoll et al Genes & Dev (2009).

[3] I. De Vlaminck et al., Nucleic Acids Research (2010).

### 1318-Pos Board B228

#### **Resolving RAD51 Filament Nucleation, Extension and Disassembly on ssDNA and dsDNA One Protein at a Time**

**Andrea Candelli, J.T. Holthausen, M. Modesti, R. Kanaar, C. Wyman, Erwin Peterman, Gijs J. Wuite.**

Homologous recombination is essential for the preservation of genome stability. The core protein in this process, RAD51, drives homology search and DNA strand exchange, processes that requires the nucleation, assembly and disassembly of a RAD51 filament on single-stranded (ss) and double-stranded (ds)DNA, coupled to ATP binding and hydrolysis. Here we show that we can characterize all these RAD51 DNA transactions on long individual DNA molecules, in real-time, at the single-protein level using a combination of single-molecule fluorescence microscopy and optical tweezers. These experiments show that the sizes of RAD51 nuclei on ssDNA vary and display a broad Poissonian distribution with an average size of 4 monomers. Filament extension tracked in time with single-protein resolution reveals that nuclei extend by one RAD51 monomer at a time with a rate independent of tension on the ssDNA. This is in contrast to force-dependent monomeric extension on dsDNA. Counting and timing individual RAD51 monomers disassembling from nucleoprotein filament on ssDNA also yields contrasting results compared with dsDNA, reflecting the difference in the underlying mechanical properties ssDNA and dsDNA based nucleoprotein filaments. Together, these results yield unprecedented quantitative insight in the mechanical rearrangement during formation and collapse of RAD51 nucleoprotein filaments.

### 1319-Pos Board B229

#### **Investigating Nucleotide Excision DNA Repair by Single-Molecule Imaging of Quantum Dot Labeled Proteins Reveals Unique Scanning Mechanisms**

**Robert Keller, Hong Wang, Mohammed A. Towheed, Bennett Van Houten, Neil M. Kad.**

How DNA repair proteins locate and repair lesions amongst a vast excess of undamaged DNA is a key question for cell survival across all kingdoms of life. Previously, using a newly developed single molecule approach we studied how the emergent properties of the first two enzymes (UvrA & UvrB) of the prokaryotic Nucleotide Excision Repair system made this lesion search possible. In this study we have introduced the next enzyme in the pathway, UvrC, which nicks the DNA backbone 5' and 3' to the pre-incision complex formed after UvrAB finds the lesion. This provides a substrate for DNA helicase II which subsequently removes the damage-containing oligonucleotide. We have cloned and expressed UvrC with an avi-tag that covalently links to biotin thus providing a conjugation moiety for quantum dot attachment. Electrophoretic mobility shift assays indicate that tagged UvrC is still able to bind DNA and AFM data suggest that UvrC is a monomer. Initial fluorescence results using our single molecule 'tightrope' approach indicate that when a color mixed population of UvrC is investigated there is little colocalization, confirming a monomer is capable of binding to DNA. The interaction of UvrC with DNA shows an interesting pattern of a short period of 1D diffusion before halting on the DNA. However, in the presence of UvrB we observe that the diffusive motion persists for the duration of attachment. Not only does this suggest that UvrB alters the search mechanism of UvrC, as it does for UvrA, but also that UvrC is capable of bringing UvrB to the DNA. Physiologically, UvrC may take advantage of the cellular excess of UvrB to enhance its diffusive search for other UvrB molecules trapped in pre-incision complexes.

### 1320-Pos Board B230

#### **Novel Conformational States in Mutator DNA Polymerases Observed Using Single-Molecule FRET**

**Johannes Hohlbein, Catherine M. Joyce, Pouya Shoolizadeh, Geraint Evans, Olga Potapova, Oya Bermek, Diego Duchillumiguis, Nigel D.F. Grindley, Achillefs N. Kapanidis.**

The remarkable fidelity of DNA polymerases depends largely on their efficient rejection of incorrect nucleotides prior to nucleotide addition. Previously, we used single-molecule FRET to examine fidelity-related conformational transitions preceding nucleotide addition by DNA polymerase I (Klenow fragment). Our experiments distinguished the open and closed conformations that predominate in the binary Pol-DNA and ternary Pol-DNA-dNTP(complementary) complexes, and showed that the unliganded polymerase is highly conformationally flexible. We also showed that ternary complexes with mismatched dNTPs or complementary ribonucleotides form novel FRET species perhaps corresponding to partially closed conformations which may act as kinetic checkpoints crucial for fidelity.

Here, we studied how amino acids proximal to the polymerase active site contribute to fidelity by examining the conformational states of polymerase derivatives that act as "mutators", i.e., have decreased fidelity. For example, an